Universidade de Aveiro Departamento de Biologia
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Ana Margarida dos Santos Valente

Red and roe deer densities and distribution in northeastern Portugal

Densidade e distribuição de veado e corço no Nordeste de Portugal

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(Ana Margarida dos Santos Valente)

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica do Doutor Carlos Manuel Martins Santos Fonseca, Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro, e sob a co-orientação científica da Doutora Rita Maria Tinoco da Silva Torres, investigadora de pós-doutoramento no Departamento de Biologia da Universidade de Aveiro e do Doutor Tiago André Lamas Oliveira Marques, investigador no *Centre for Research into Ecological & Environmental Modelling* na Universidade de St Andrews.

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Ao Reino Maravilhoso

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palavras-chave

Ungulados, Portugal, monitorização, amostragem de distâncias, modelação espacial, modelos aditivos generalizados, métodos indiretos

resumo

A monitorização de ungulados constitui um passo essencial no desenvolvimento de estratégias de gestão. Desde os anos 70, quando os esforços para a conservação e gestão destas espécies permitiram a sua expansão, até aos dias de hoje, a gestão de habitats e espécies tem tido um papel central na ecologia. A ampla distribuição atual dos ungulados selvagens e as suas elevadas abundâncias na Europa provocam danos nos ecossistemas, que têm que ser geridos com base em conhecimento científico. Em Portugal a monitorização das populações de ungulados, bem como o estudo da sua ecologia encontra-se ainda numa fase inicial, no entanto têm sido desenvolvidos avanços significativos no conhecimento das populações de ungulados no nordeste transmontano. Neste trabalho foram estimadas densidades de veado e corço através do *distance sampling* aplicado a transectos lineares com contagem de excrementos. A densidade de veado no Parque Natural de Montesinho (PNM) foi de 3.05 ind./100 ha (IC a 95%: 2.05 – 4.54) dividido em duas sub-áreas: Serra de Montesinho (SM) com 1.23 ind./100 ha e *Lombada National Hunting Area* (LNHA) com 5.23 ind./100 ha. As densidades de corço foram estimadas com recurso a uma metodologia espacial desenvolvida recentemente, os *Density Surface Models* (DSMs – baseados no *distance sampling*) que permitem relacionar as densidades populacionais com as variáveis espaciais escolhidas de acordo com a ecologia da espécie. As densidades de corço foram estimadas para o PNM e para a Serra da Nogueira (SN) apresentando uma densidade global de 3.01 ind./100 ha (IC a 95%: 2.34 – 3.87): SM com 3.74 ind./100 ha, LNHA com 1.59 ind./100 ha e SN com 3.62 ind./100 ha. Adicionalmente este método permite construir um mapa de distribuição de abundâncias ao longo da área de estudo, o que é particularmente útil ao comunicar os resultados aos responsáveis pela gestão das áreas protegidas. Do ponto de vista ecológico, as densidades de corço aumentaram à medida que a distância às estradas aumentou, mostrando, surpreendentemente, uma redução na densidade à medida que a distância às populações humanas aumentou. Tratando-se de uma espécie-presa do lobo-ibérico, as áreas de abrigo revelaram-se importantes para o corço. A análise espacial confirmou que os DSMs são um método robusto para estimar densidades de ungulados e analisar a sua ecologia. Estudos futuros são essenciais para identificar as necessidades ecológicas do veado e do corço, bem como para avaliar oscilações nas densidades ao longo dos anos, para que seja possível estabelecer planos de gestão que permitam mitigar os danos causados por estas espécies.

keywords Ungulates, Portugal, monitoring, *distance sampling*, spatial modelling, generalized additive models, indirect methods, deer management

abstract Monitoring ungulates is a major challenge to perform management strategies, either back in the 70's to enable their conservation that lead to their great recovery, as to manage their actual expansion. Their current wide range distribution and high densities across Europe promotes damages in ecosystems that need to be handled based on scientific knowledge. In Portugal ungulate monitoring and ecology is still in an early stage, however efforts have been made to gather valid information on north-eastern ungulate populations. In this work density of red and roe deer were estimated coupling line transects to perform pellet group counts with a *distance sampling* approach. The density of red deer estimated for Montesinho Natural Park (MNP) was 3.05 ind./100 ha (95% CI: 2.05 – 4.54), splitted in two sub-areas: *Serra de Montesinho* (SM) with 1.23 ind./100 ha and Lombada National Hunting Area (LNHA) with 5.23 ind./100 ha. Roe deer densities were estimated with recourse to a spatial methodology recently developed, the Density Surface Models (DSMs – with a *distance sampling* framework), which enables the assessment of the relationships between animal's density and spatial variables selected according to species ecological requirements. As well roe deer densities were estimated for MNP and *Serra da Nogueira* (SN) with a global density of 3.01 ind./100 ha (95% CI: 2.34 – 3.87): SM with 3.74 ind./100 ha, LNHA with 1.59 ind./100 ha and SN with 3.62 ind./100ha. Furthermore this approach enables the drawing of an abundance distribution map across the study area, especially useful when communicating results to wildlife managers. Roe deer densities showed to increase as distance to roads increased, while surprisingly shown an increase as distance to human populations decreased. As expected, cover areas shown its importance for roe deer, a prey species for Iberian wolf. The spatial analysis confirmed that DSMs represent a good approach to estimate ungulate densities, and should be encouraged in future works. Future studies are mandatory to assess red and roe deer ecological requirements and evaluate trends over the years, in order to stablish management plans to handle the damages caused by these species.

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Chapter I

Introduction

1. Introduction

In the last decades ungulate populations have experienced an increase in density and distribution area throughout Europe and North America (Rooney 2001; Apollonio et *al.* 2010). One of the most abundant and widespread ungulate species in Europe is roe deer, *Capreolus capreolus* (Apollonio et *al.* 2010). Likewise, the Iberian red deer *Cervus elaphus hispanicus* has registered a significant expansion in Iberian Peninsula (Vingada et *al.* 2010). It was just a century ago that these species were almost facing extinction in Portugal. A sequence of reintroduction programs during the 90's, and natural dispersion from Spanish populations, led to stable roe and red deer populations, which are now common and widespread throughout Portugal (Vingada et *al.* 2010). Such increase has triggered the need for urgent conservation and management measures (Apollonio et *al.* 2010). But the implementation of such management practices require effective and robust monitoring schemes. When compared to the European overabundance scenario, Portugal has a far less concerning situation (Santos 2009; Carvalho 2011; Valente et *al.* 2014). Nevertheless, it is timely to monitor current population and trends in order to anticipate and prevent high deer densities (for a review see Putman et *al.* 2011). Furthermore, monitoring programs can potentiate the exploitation of ungulates as a boost for eco-tourism, which could be a significant contribute to a weakened economy in northeastern Portugal, driven mainly by the rural exodus in the last decades.

So far the initial steps regarding ungulate populations in northeastern Portugal have been taken, from an ecological point of view (Torres et *al.* 2011 – roe deer *Capreolus capreolus* and Torres et *al.* 2014 red deer *Cervus elaphus*) to monitoring populations abundance and densities (Santos 2007; Santos 2009; Carvalho 2011 - red deer and Valente et *al.* 2014 - roe deer). Deer monitoring can be achieved through direct methodologies, where the animals are directly observed, or indirect methods, where density estimation is based on signs left by animals, namely pellet groups. The selection of the method to implement should take into account the main aim of the study, the logistical and financial resources available, the ecology of the study species and the management questions to be answered (Mayle et *al.* 1999). Furthermore, long-term monitoring programs should have a standardized method to reduce method bias and improve estimates accuracy.

Unlike previous studies (Santos 2007; 2009 and Carvalho 2011) in this area, our work is based on an indirect methodology, through pellet group counts. However, both authors based their data analysis in a *distance sampling* framework, as occurred in this study.

Despite getting density and abundance estimates wildlife managers increasingly need to obtain more than numbers from their surveys (Katsanevakis 2007). Taking this into account, the ecological relations between environmental conditions and roe and red deer presence were assessed by Torres et *al.* (2011; 2014), respectively. Still, with the development of spatial modelling it became reasonable to attempt to relate animal density, rather than presence/absence, with spatial variables thought to influence species. This is currently achievable with the distance data collected in the field and the environmental variables available through Geographic Information Systems (GIS), namely through ArcMAP (version 10.1) software. Hence, in the third chapter of this work, rather than only applying a design-based approach to account for detectability, a model-based inference was achieved through Density Surface Modelling (DSM) for roe deer. The spatial modelling was applied not only to obtain more sensitive estimates *per* area as also to provide important observations regarding roe deer abundance distribution revealing valuable ecological patterns. This approach enables also the construction of an abundance distribution map throughout our study area. Spatial modelling of distance data will be based on an indirect methodology (pellet group counts), achieved in a two-stage approach: in the first-stage there will be a fit of a detection function; in the second-stage will take place the spatial modelling of the distance data with the chosen variables thought to influence roe deer density. In fact, to our knowledge, this is the first time that such an approach is applied for an ungulate species in Europe.

In the fourth and last chapter we provide a global discussion of the results, as well as the main conclusions both ecological and methodological. Simultaneously, future work recommendations will be made according to the new scientific data brought along the previous chapters. Our specific objectives are described in the next subsection, laying the structure for the thesis.

1.1. Objectives

1) Density and abundance estimation of red deer through standard *distance sampling* implemented in DISTANCE 6.0 software (Thomas et *al.* 2010);

2) Evaluation of the role of covariates in the detectability function for the two species (red and roe deer) through Multiple Covariate Distance Sampling (Marques et *al.* 2007);
3) Density and abundance estimation of roe deer through standard *distance sampling* methods in R v.3.0.0 (R Development Core Team 2013) with *Distance* package (Miller 2014);
4) Evaluation of the influence of the chosen variables (proximity to roads, proximity to human settlements, percentage of cover areas – coniferous and deciduous forests - and geographical coordinates) in roe deer density and abundance in R v.3.0.0 (R Development Core Team 2013) through *dsm* package (Miller et *al.* 2013);
5) Construction of an abundance map for the survey area and abundance estimations through density surface modelling in R v.3.0.0 (R Development Core Team 2013) with *dsm* package (Miller et *al.* 2013);

1.2 References

Apollonio, M., Andersen, R. & Putman, R. (2010) European Ungulates and Their Management in the 21st Century. Cambridge: Cambridge University Press. 618 p.

Carvalho, J. (2011) Monitorização da população de veado (*Cervus elaphus* L.) na Zona de Caça Nacional da Lombada, Nordeste Transmontano: Época 2010/2011. Relatório de estágio. Universidade de Aveiro, Aveiro.

Katsanevakis, S. (2007) Density surface modelling with line transect sampling as a tool for abundance estimation of marine benthic species: the *Pinna nobilis* example in a marine lake. *Marine Biology*, *152*(1), 77-85.

Marques, T. A., Thomas, L., Fancy, S. G., & Buckland, S. T. (2007) Improving estimates of bird density using multiple-covariate distance sampling. *The Auk*, *124*(4), 1229-1243.

Mayle, B. A., Peace, A. J., Gill, R. M. (1999) How many deer? A field guide to estimating deer population size. Edinburgh: Forestry Comission Publications. 96 p.

Miller, D. L., Rexstad, E., Burt, L., Bravingtion M. V., Hedley, S. (2013) dsm: Density surface modelling of distance sampling data. R package version 2.1.3. URL: http://CRAN.R-project.org/package=dsm

Miller, D. L. (2014) Distance: A simple way to fit detection functions to distance sampling data and calculate abundance/density for biological populations. R package version 0.8.0. http://CRAN.R-project.org/package=Distance

Putman, R., Watson, P., & Langbein, J. (2011) Assessing deer densities and impacts at the appropriate level for management: a review of methodologies for use beyond the site scale. *Mammal Review*, *41*(3), 197–219.

R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

Rooney, T. P. (2001) Deer impacts on forest ecosystems: a North American perspective. *Forestry*, *74*(3), 201–208.

Santos, J. P. (2007) Dinâmica populacional do veado (*Cervus elaphus*) no Nordeste transmontano. 63pp. Universidade de Aveiro. Aveiro.

Santos, J. P. (2009) Estudo populacional do veado (*Cervus elaphus* L.) no Nordeste Transmontano. Tese de Mestrado em Ecologia, Biodiversidade e Gestão de Ecossistemas. Universidade de Aveiro, Aveiro

Thomas, L., Buckland, S. T., Rexstad, E. A., Laake, J. L., Strindberg, S., Hedley, S. L., Bishop, J. R., Marques, T. A. & Burnham, K. P. (2010) Distance software: design and analysis of distance sampling surveys for estimating population size. *Journal of Applied Ecology*, *47*, 5-14.

Torres, R. T., Santos, J., Linnell, J. D. C., Virgós, E., & Fonseca, C. (2011) Factors affecting roe deer occurrence in a Mediterranean landscape, Northeastern Portugal. *Mammalian Biology*, *76*(4), 491–497.

Torres, R. T., Santos, J., Fonseca, C. (2014) Factors influencing red deer occurrence at the southern edge of their range: A Mediterranean ecosystem. *Mammalian Biology*. *79*(1), 52-57.

Valente, A. M., Fonseca, C., Marques, T. A., Santos, J. P., Rodrigues, R. & Torres, R. T. (2014) Living on the edge: roe deer (*Capreolus capreolus*) density in the margins of its geographical range. *PloS one, 9*(2).

Vingada, J., Fonseca, C., Cancela, J., Ferreira, J., Eira, C. (2010) Ungulates and their management in Portugal. In: European Ungulates and their management in the 21st century. pp.618.

Chapter II

Estimating the density of an expanding deer population: an example with red deer in Mediterranean habitats

Estimating the density of an expanding deer population: an example with red deer in Mediterranean habitats

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2.1 Abstract

Ungulate populations have experienced an increase in number and distribution throughout Europe in the last decades. One of the ungulates that have registered a significant expansion in Iberian Peninsula was the Iberian red deer *Cervus elaphus*. As red deer population increases, the negative impacts that they can cause in the ecosystem tend inevitably to increase. Here, we sought a method to estimate density and abundance of red deer that is reliable, flexible to local environmental conditions, useful at multiple temporal and spatial scales and that can be easily used in different management scenarios. We estimated red deer population density in a Mediterranean environment located at northeastern Portugal (*Lombada National Hunting Area* - LNHA and *Serra de Montesinho* - SM), using pellet group counts coupled with *distance sampling* to account for detectability. The estimated red deer density using a stratified detection function was 5.81 ind./100 ha for LNHA and 1.34 ind./100 ha for SM (95% CI: 3.65 – 9.25 and 0.74 – 2.42, respectively). For the entire area, the estimated density was 3.38 ind./100 ha (95% CI: 2.18 – 5.24). By using pellet group counting, coupled with *distance sampling*, all the distance sampling assumptions were met, therefore this is an effective method for monitoring increasing red deer populations in Portugal. We encourage other agencies to develop a standardized protocol to implement across red deer range in Portugal to maintain consistent data collection and analysis for comparisons over time, thereby helping to assess population trends and determine appropriate harvest levels for this desirable game species range-wide.

Keywords: Cervidae, Distance Sampling, deer density, pellet group counting, Iberian Peninsula.

2.2 Introduction

Ungulate populations have been steadily increasing in range and numbers throughout Europe in the last decades (Apollonio et al., 2010). The paradigm regarding ungulates has changed: from a decrease in numbers and distribution in the 19th century (Côte et al., 2004) to the actual scenario of expanding populations over Europe (Apollonio et al., 2010). As ungulate populations increase, the impacts that they can cause in the ecosystem tend inevitably to increase (see Putman et al., 2011a for a review). Ungulates are particularly difficult to monitor (Putman et al., 2011a) but effective monitoring programs are pivotal to cope with their current expansion. Morellet et al. (2007) proposed to monitor a set of indicators of ecological change that over time will capture the interactions between the population and its habitat as a basis for adaptive management in order to achieve specific predefined goals. The idea behind the indicators of ecological change is very well rooted on the density-dependence concept and that the temporal trends of these indicators reflect the demographic course of the population (Morellet et al., 2007). These authors described and validated a series of parameters that change according to roe deer population size, which include population abundance, animal performance (*e.g.* female reproductive success) and the interaction between the population and its habitat (*e.g.* browsing index), among others.

Regarding the estimation of the abundance of ungulate populations, a wide variety of techniques have been used (Apollonio et al., 2010). Among the indirect methods, pellet group counting has been widely used to estimate deer densities throughout the world (Marques et al., 2001; Smart et al., 2004) and while many authors have argued against it (Morellet et al., 2007), others have recommended it, claiming that it can determine population size and trends (Acevedo et al., 2008). Indirect methods are usually preferred in woodland habitats since direct ones are often not feasible or they are potentially biased by the animal's detectability. Advantages in using pellet group counts include being easy to implement over large areas, requiring low financial and logistical resources and being especially useful in areas with low visibility (Marques et al., 2001; Smart et al., 2004). Pellet group counting coupled with Distance sampling has allowed to obtain pellet group densities and, eventually estimate population densities when defecation and production rates are known (Smart et al., 2004; Valente et al., 2014). *Distance sampling* (Buckland et al., 2001) has been widely used to estimate density and abundance of a variety of ungulate species *e.g.* sika deer

Cervus nippon (Marques et al., 2001), sambar *Cervus unicolor* and muntjac *Muntiacus muntjak* (Jathanna et al., 2003); roe deer *Capreolus capreolus* (Focardi et al., 2001; Acevedo et al., 2010; Herrero et al., 2013), Iberian wild goat *Capra pyrenaica* and mouflon *Ovis aries* (Torres et al., 2014a).

One of the ungulates that have registered a significant expansion in Iberian Peninsula is the Iberian red deer *Cervus elaphus hispanicus* (Vingada et al., 2010). It was just a century ago that this species was almost facing extinction in Portugal. A sequence of reintroduction programs during the 90's, and natural dispersion from Spanish populations, led to stable red deer populations, which are now common and widespread throughout Portugal (Vingada et al., 2010) with the most representative populations located near the border areas with Spain. Accordingly, it is therefore timely to evaluate red deer population size, since it is fundamental to determine population trends as well to identify management and conservation needs. However, in Portugal, conservation and management of red deer populations are still on their initial steps (Torres et al., 2012; Torres et al., 2014b).

Here, we sought a method to assess population trends considering temporal variation to detect fluctuations on population density, useful at multiple spatial scales that can be easily used by park rangers across the entire red deer range in Portugal. We propose this approach as a way to monitor the population density section of the above-mentioned indicators of ecological change. We estimated population density of red deer using pellet group counts coupled with *distance sampling* to account for detectability. Simultaneously, while ensuring geographic stratification to obtain more sensitive estimates *per* area, covariates were collected to assess their impact on the detectability of pellet groups. This study will serve as an initial reference point, which is intended to be used to assess red deer population trends, serving as a tool for long-term monitoring projects. This scientific basis will help to guide management plans in an attempt to recognize and mitigate deer impacts and promote an adequate management.

2.3. Methods

2.3.1. Study area

The study was carried out in Montesinho Natural Park (6º30'-7º12'W, 41º43'-41º59'N) part of the European Union's Natura 2000 Network, covering an area of 75,000 ha (Figure 1). The terrain consists of rolling hills with elevation ranges from 438 to 1,481m. The climate is Mediterranean with the mean annual temperature varying between 3ºC in the coldest month and 21ºC in the warmest month and mean precipitation between 600 and 1,500 mm. The vegetation is diverse, characterized mainly by oak (*Quercus pyrenaica*, *Quercus rotundifolia*, *Quercus suber*), sweet chestnut (*Castanea sativa*) and maritime pine (*Pinus pinaster*). The shrub vegetation is dominated by heather (*Erica* spp.), gum rockrose (*Cistus ladanifer*) and furze (*Ulex europaeus* and *Ulex minor*). Other mammals present are the Iberian wolf (*Canis lupus signatus*), wildcat (*Felis silvestris*), wild boar (*Sus scrofa*) and the sympatric roe deer (*Capreolus capreolus*), among others. The study area is crossed by some rivers and includes small villages with a low human presence (9.5 people per km^2).

2.3.2. Field methods and sampling design

The survey area was divided in 2 geographic strata: *Serra de Montesinho* (SM: 24,800 ha) and *Lombada National Hunting Area* (LNHA: 20,830 ha). This was done to provide straightforward separate estimates by relevant management areas (red deer is hunted in LNHA during September - hunting bag is 5-7 animals although in some years it is not filled - but not in SM). Transect location followed a systematic random design, which is considered more reliable and provides a better coverage of the area, ensuring that transects were representative of the whole area. In total, there were 49 transects: 27 in LNHA and 22 in SM (Figure 1). Each transect was 1,000m long; in order to maximize spatial coverage and to mitigate sampling dependence, sampling plots were 100m (of effective sampling) spaced 200m (without prospection) along the line. Fieldwork was performed from January 2012 to October 2013 (2012: January and November; 2013: January, February and October). From the beginning of the transect, and using a handheld Global Positioning System (GPS) unit and a compass, it was possible to follow a straight line. A rope was used to facilitate the progress in a straight line in a woodland habitat, ensuring the prospection of 1m from each side of the line and to guarantee accurate measurements of perpendicular distance of the pellet groups to the center of the transect. Whenever a pellet group was detected, the perpendicular distance from the center of each pellet group to the transect line was recorded with a measuring tape. To minimize the risk of counting one spread group as two pellet groups we considered only pellet groups with six or more individual pellets (produced at the same defecation event, identified for similar size, shape, texture and color) (Mayle et al., 1999). Four observation level covariates were collected: i) the size of the pellet group (medium: between 6 to 40 individual pellets vs. large: more than 40 individual pellets); ii) dispersion of the group (aggregated vs. scattered); iii) the type of habitat around the pellet group (open vs. close); and iv) detectability, *i.e.* if the pellet group was clearly visible or not. Two persons always performed fieldwork and one of the observers was constant throughout all fieldwork.

2.3.3. Density estimation

Taking into account habitat conditions and the available resources, an indirect density estimation method was implemented. Animal density was estimated within a distance sampling framework. The distance sampling approach relies on the assumption that detection of animals/objects is not certain (Buckland et al., 2001). By modelling the decrease in detectability with increasing distance from the transect line there is a fit of a detection function (Buckland et al., 2001; Miller et al., 2013). The detection function, *g(x)*, gives the probability ($\hat{p}$) of detecting an object in a distance *y* from the center of the transect line. This function can then be used to estimate the detection probability *P* within the covered area, as

$$P = \int_0^w g(x)\pi(x)dx$$

where *w* is a truncation distance and π(x) represents the distribution of available distances. This distribution is assumed to be uniform by design, given the random placement of the transect lines. The estimate of *P* leads to a density estimator as follows. Given the n_i detected pellet groups in stratum *i*, an animal density estimate is given by

$$\hat{D}_i = \frac{\hat{D}_i^p}{\hat{\alpha}\hat{\beta}} = \frac{n_i}{2L_i w \hat{P}_i \hat{\alpha}\hat{\beta}}$$

where L_i represents the total on-effort line length in stratum *i* (*i*=1,2), P_i represents the detection probability of a group within the covered area in stratum *i*, α represents the production rate: how many pellet groups produces a deer *per* day; and β the decay of pellet groups: how many days takes a pellet group not to be recognized as a group (> of 6 individuals). Note that the animal density estimator is just the pellet group density (D^p) estimator, divided by the required production and decay rates. This notation implicitly conveys the assumption that both of these are constant across strata. The global density (D) estimate is obtained as a weighted average of stratum specific estimates, with stratum's areas as weights (Buckland et al., 2001), *i.e.*

$$\hat{D} = \frac{\sum_{i=1}^{3} \hat{D}_l A_i}{\sum_{i=1}^{3} A_i}$$

The variance of the stratum specific estimates is obtained via the delta method, by combining the variances of the random components in the estimator defined above (see Buckland et al., 2001 for details).

In this study the values of α and β were obtained from two different sources. The mean number of days that a pellet group takes to disappear, β, was assumed to be 227±24 days, a value provided by Torres et al. (2013) for red deer in MNP. The production rate, α, was considered to be 25, value estimated in the UK (Mayle et al., 1999). We address the plausibility of these values and consequences of bias in these parameters in the final density estimates in the discussion.

2.3.4. Data analysis

Distance 6.0 software (Thomas et al., 2010) was used to analyze the data. In order to evaluate the role that covariates can have in the detection function (Marques et al., 2007) and to assess if a more parsimonious model could be obtained including habitat type, amount of dispersion of pellet group and pellet group size as covariates, Multiple Covariate Distance Sampling (MCDS) analyses were used. Data were right-truncated by 5%, which is a standard procedure (Marques, 2004; Ward et al., 2004), to avoid fitting spurious bumps in the tails of the detection function (Marques et al. 2001), therefore observation beyond 90 cm were discarded. Three models, *half-normal*, *uniform* and *hazard-rate*, were fitted against the data, with the available adjustment terms (cosine, simple polynomial and hermite polynomial) to assess which resulted in a more parsimonious detection function (Buckland et al., 2001). The evaluation of the model is based mainly on the lowest AIC value (Burnham and Anderson, 1984), although ΔAIC, *goodness-of-fit* test and histogram appearance were also inspected. Chi-squared and Cramer von-Mises goodness-of-fit tests were used as absolute measures of fit to evaluate the adequacy of the final model chosen for inference.

2.4. Results

In a total of 19,600 m of effort (SM – 8,800 m; LNHA – 10,800 m) 527 pellet groups were recorded. The number of records monotonically decreased with distance, as expected, and no problems were apparent from visual inspection of the data. Nevertheless a broad shoulder was present, revealing a wide number of observations near the center of the transect. The model that better fitted distance data was a half-normal model for LNHA (a), and a uniform model for SM (b), both with cosine adjustment term (Figure 2). The separate analysis corresponded to the most parsimonious model, although a pooled analysis was carried out, with the uniform model with cosine adjustment term corresponding to a better fit (Figure 3). Contrariwise to the expected, none of the covariates contributed to a better fit of the model, and thus the model with the distance alone was selected for further inference. The goodness-of-fit p-values for such model were 0.008 (LNHA) and 0.035 (SM) for the Cramer von-Mises test and 0.01 (LNHA) and 0.4 (SM) for the chi-squared test (Table 1). The density estimates *per* stratum were 5.81 ind./100 ha (95% CI of 3.65 to 9.25) for LNHA and 1.34 ind./100 ha (95% CI of 0.74 to 2.42) for SM and the global density estimate was 3.38 ind./100 ha (95% CI of 2.18 to 5.24) and a coefficient of variation of 22.18% (Table 2).

2.5. Discussion

An important step forward concerning wildlife monitoring has been the link between research and management. This has allowed the application of rigorous scientific methods to wild populations estimations therefore increasing the quality of the results and consequently, potentially, the sustainable management of wild populations.

Red deer densities are higher in LNHA when compared with SM. This was expected as this population results from natural dispersion from the Spanish border populations and the first nucleus of red deer populations in MNP were originally established in LNHA and from there it continued expanding (Santos 2009). In our study area, red deer densities were 3.38 ind./100 ha (LNHA: 5.81 ind./100 ha and SM: 1.34 ind./100). Santos (2007; 2009) and Carvalho (2011), using direct animal counts with distance sampling, estimated that red deer densities in LNHA were 3.26 ind./100 ha and 1.75 ind./100 ha, respectively. Even if our density estimates are slightly higher than the ones estimated previously, it is difficult to compare our estimates with these two other studies because they are focused on direct counts of the animals either from transect lines or spotlighting surveys while we have used pellet group counts (indirect counts). Plus, the previous authors only surveyed the north part of the LNHA whereas we surveyed the whole area. Consequently, caution should be taken when comparing with previous studies, which used different methods, as comparison of estimates with different methods is often of limited validity.

Here we used an indirect method based on pellet groups coupled with distance sampling to estimate red deer density and therefore is based on the distance sampling assumptions. *Distance sampling* is based on four key assumptions: i) objects (pellet groups in this case) on the transect line are always detected. It is unlikely that the pellet groups lying on the line are missed, but even if they were, as we are looking for static objects in a very narrow transect, the g(0)=1 assumption would suffer at worst minor violations; ii) sampling is instantaneous and animals do not move in response to observer before being detected; because pellets are immobile this assumption holds with certainty; iii) perpendicular distances to the centre of the transect line are accurate and iv) obtaining estimates for the parameters of the detection function by maximum likelihood requires that detection events are assumed independent, but methods are very robust to the failure of this assumption (Burnham and Anderson, 1984). The base line is that if these assumptions are met, estimates tend to be unbiased, even in the presence of heterogeneity in detection probability. This way, by using pellet group counting, coupled with *distance sampling*, all the distance sampling assumptions are met, which is obviously a big advantaged when compared with the use of direct methods and distance sampling. Nevertheless we are aware that bias can arise from the use of an indirect methodology such as pellet group counting. The need of conversion factors (*e.g.* decay rate and production rate of red deer) can actually interfere in the estimated value and cause an increase in the CV (Plumptre, 2000). Here we expect minimal bias from the decay rate as it was previously calculated for red deer and in the region of interest (Torres et al., 2013). Since disappearance days can vary among habitats, the use of a site-specific value for each dominant habitat, over the mean value, should be assessed in future works. Laing et al (2003) has estimated a decay rate of 295±31 days for red deer in Scotland, which, if used, would result in lower density estimates. However, since we have a site-specific value of disappearance days we chose to use Torres et al. (2013) over Laing et al. (2003). Still, the main problem with our estimate concerns the use of a production rate obtained elsewhere, specifically in the UK in the 2000's (Mayle et al., 1999). Furthermore, the value used does not have a variance or standard error associated, which means that the reported variance of density estimates ignores a potential source of variation. However, a clear advantage of the modular form of the estimator used is that, as soon as a production rate and corresponding standard error are obtained for our region, the density estimates and corresponding variances reported here could be easily updated. Obtaining such production rate should therefore be a major goal for the effective management of these populations.

Surprisingly, none of the covariates collected had an impact on the detectability of pellet groups. The detection function comprising only the perpendicular distance led to a more parsimonious model that better explained detectability. This reflects the fact that *distance sampling* pooling strength property is strong and that sometimes MCDS provides no additional practical gain beyond conventional *distance sampling*. Nonetheless, under certain circumstances MCDS can be used to reduce variance estimates, by explaining some of the variance in detectability. MCDS might also allow less biased estimates of density. In fact, covariate influence on detectability might be interesting in itself. However, we encourage researchers to collect data regarding covariates that are believed to have an impact in the detectability of the study objects, since they can have an important role in the construction of the detection function of the distance data (Marques et al., 2007).

From a practical point of view, our method has several advantages: it is cheap, easy to implement over large areas, is useful in concealing habitats, can be easily applied by park rangers, which is particularly helpful in gathering data for a long-term. This approach, applied over the years, will allow tailoring management strategies in order to adjust them to demographic data, and to assess the results of management measures applied throughout the monitoring plan. Urbanek et al. (2012) showed that pellet group counting coupled with distance sampling provides less biased estimates (when accurate decay and production rates are used) and was 88% cheaper than aerial surveys. When comparing with direct methods, pellet group counting can be performed any time during the year and does not need elaborate equipment neither professional biologist to perform field work (Marques et al., 2001). While we are aware that distance sampling data analysis is rather complex, it is balanced by gains in logistical, financial, and analytical efficiency, with the added benefit of precise and comparable abundance estimates. In future, there is a need to evaluate our methodology against a reference method as capture-mark-recapture. However, at the present due to logistical restrains (*e.g.* no enclosures with available populations) it was not possible.

Even though reasonable results were obtained, the relation between animal density and spatial variables is a step forward particularly in wildlife management (Miller et al., 2013). The Density Surface Models (DSM) will allow the identification of ecological relevant variables that can constrain or promote animal populations (Miller et al., 2013). Hence, being aware of which factors contribute to the presence and density values of a species or population in a specific area will allow spatial and temporal predictions extremely useful for accurate management strategies. This should be a major goal in future studies regarding red deer in our study area. Nevertheless, estimation of population size should not be considered the only requirement to monitor red deer populations and for sure managers need other information to be considered and monitored (*e.g.* habitat composition, vegetation and agriculture impacts, vehicle collisions, among others). However, here we propose an approach as a way to monitor the population abundance compartment of ecological indicators to change as suggested by Morellet et al. (2007).

Although future steps are mandatory, particularly in collecting additional data to inform the relationship between ungulates and habitat, this study enhances the need of a continued strategy of monitoring taking into account the negative impacts arising from an expanding population. Gathering knowledge on the population size coupled with the understanding of ecological processes underlying fluctuations in densities, and long-term monitoring projects, should be a priority for developing management programs.

We encourage other agencies to set up across their study areas the protocol used in this study so we could have standard methods throughout red deer range in Portugal, easing the task to estimate densities across temporal and spatial scales. We expect that their implementation would enable managers to survey a much larger proportion of the red deer range, thereby helping to assess population trends.

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2.7. References

Acevedo P, Ferreres J, Jaroso R, Durán M, Escudero MA, Marco J, Gortázar C (2010) Estimating roe deer abundance from pellet group counts in Spain: An assessment of methods suitable for Mediterranean woodlands. Ecol Indic 10(6): 1226-1230

Acevedo P, Ruiz-Fons F, Vicente J, Reyes-García a. R, Alzaga V, Gortázar C (2008) Estimating red deer abundance in a wide range of management situations in Mediterranean habitats. J Zool 276(1):37–47.

Apollonio M, Andersen R, Putman R (2010) European ungulates and their management in the 21st century. Cambridge. Cambridge; pp.604.

Buckland ST, Anderson DR, Burnham KP, Laake JL, Borchers DL, Thomas L (2001) Introduction to Distance Sampling. Oxford Uni. Oxford; pp.432.

Burnham KP, Anderson DR (1984) The need for distance data in transect counts. J Wildl Manage, 1248- 1254.

Carvalho J (2011) Monitorização da população de veado (*Cervus elaphus* L.) na Zona de Caça Nacional da Lombada , Nordeste Transmontano: Época 2010/2011. Bachelor degree. Aveiro University, Portugal.

Côté SD, Rooney TP, Tremblay J, Dussault C, Waller DM (2004) Ecological impacts of deer overabundance. Annu Rev Ecol Evol S 35:113–147.

Focardi S, Isotti R, Tinelli A (2002) Line transect estimates of ungulate populations in a mediterranean forest. J Wildl Manage 66(1):48–58.

Garel M, Bonenfant C, Hamann J-L, Klein F, Gaillard J-M (2010) Are abundance indices derived from spotlight counts reliable to monitor red deer *Cervus elaphus* populations? Wildl Biol 16(1):77–84.

Herrero J, Torres RT, Prada C, García-Serrano A, Gimenez-Anaya A, Fernández-Arberas O (2013) Sustainable monitoring of roe deer in public hunting areas in the Spanish Pyrenees. For Syst *22*(3): 456-462.

Horsley SB, Stout SL, DeCalesta DS. (2003). White-tailed deer impact on the vegetation dynamics of a northern hardwood forest. Ecol Applic 13(1): 98-118.

Jathanna D, Karanth KU, Johnsingh AJT. (2003). Estimation of large herbivore densities in the tropical forests of southern India using distance sampling. J Zool, 261(3): 285-290.

Mayle BA, Peace AJ, Gill RMA (1999) How many deer? A field guide to estimating deer population size. Edinburgh: Forestry Commission Field Book 18. Forestry Commission.

Marques FFC, Buckland ST, Goffin D, Dixon CE, Borchers DL, Mayle BA, Pease AJ (2001) Estimating deer abundance from line transect surveys of dung: sika deer in southern Scotland. J Appl Ecol 38(2):349–363.

Marques TA (2004) Predicting and correcting bias caused by measurement error in line transect sampling using multiplicative error models. Biometrics 60(3): 757-763.

Marques TA, Thomas L, Fancy SG, Buckland ST (2007) Improving estimates of bird density using Multiple- Covariate Distance Sampling. The Auk. 124(4):1229.

Miller DL, Burt ML, Rexstad EA, Thomas L (2013) Spatial models for distance sampling data: recent developments and future directions. Methods Ecol Evol 4(11): 1001-1010.

Morellet N, Gaillard JM, Hewison AJM, Ballon P, Boscardin Y, Duncan P, Klein F, Maillard D (2007) Indicators of ecological change: new tools for managing populations of large herbivores. JAppl Ecol 44: 634–643.

Laing SE, Buckland ST, Burn RW, Lambie D, Amphlett A. (2003). Dung and nest surveys: estimating decay rates. J Appl Ecol 40(6): 1102-1111.

Putman R, Apollonio M, Andersen R (2011a) Ungulate management in Europe: problems and practices. Cambridge University Press, Cambridge.

Putman R, Langbein J, Green P, Watson, P (2011b) Identifying threshold densities for wild deer in the UK above which negative impacts may occur. Mammal Rev 41(3): 175-196.

Putman R, Watson P, Langbein J (2011c) Assessing deer densities and impacts at the appropriate level for management: a review of methodologies for use beyond the site scale. Mammal Rev 41(3):197–219.

Plumptre AJ. (2000). Monitoring mammal populations with line transect techniques in African forests. J Appl Ecol 37(2), 356-368.

Santos JP (2007) Dinâmica populacional do veado (*Cervus elaphus*) no Nordeste transmontano. 63pp. University of Aveiro. Aveiro.

Santos JP (2009) Estudo populacional do veado (*Cervus elaphus L.*) no Nordeste Transmontano. 97pp. MSc thesis. University of Aveiro. Aveiro.

Seiler A. (2005). Predicting locations of moose–vehicle collisions in Sweden. J Appl Ecol 42(2): 371- 382.

Smart JC, Ward AI, White PC (2004) Monitoring woodland deer populations in the UK: an imprecise science. Mammal Rev 34: 99-114.

Thomas L, Buckland ST, Rexstad E, Laake J, Strindber S, Hedley SL, Bishop JRB, Marques T, Burnham KP (2010) Distance software: design and analysis of distance sampling surveys for estimating population size. J Appl Ecol 47(1):5–14.

Torres RT, Virgós E, Santos J, Linnell JD, Fonseca C (2012) Habitat use by sympatric red and roe deer in a Mediterranean ecosystem. Anim Biol 62, 351-366.

Torres RT, Santos J, Fonseca C (2013) Persistence of roe (*Capreolus capreolus*) and red (*Cervus elaphus*) deer pellet-groups in a Mediterranean mosaic landscape. Wildl Biol Pract 9(3):7–18.

Torres RT, Santos J, Fonseca C (2014a) Factors influencing red deer occurrence at the southern edge of their range: A Mediterranean ecosystem. Mammal Biol 79(1), 52-57.

Torres RT, Herrero J, Prada C, Garcia-Serrano A, Fernandez-Arberas O, Garcia-Post R (2014b) Estimating the population density of Iberian wild goat *Capra pyrenaica* and mouflon *Ovis aries* in a Mediterranean forest environment. For Syst 23(1): 36-43.

Urbanek RE, Nielsen CK, Preuss TS, Glowacki GA (2012) Comparison of aerial surveys and pellet- based distance sampling methods for estimating deer density. Wild Soc Bull 36(1): 100-106.

Valente AM, Fonseca C, Marques TA, Santos JP, Rodrigues R, Torres RT (2014) Living on the edge: roe deer (*Capreolus capreolus*) density in the margins of its geographical range. PloS One *9*(2).

Vingada J, Fonseca C, Cancela J, Ferreira J, Eira C (2010) Ungulates and their management in Portugal. In: European Ungulates and their management in the 21st century. pp.618.

Ward AI, White PC, Critchley CH (2004) Roe deer *Capreolus capreolus* behaviour affects density estimates from distance sampling surveys. Mam Rev 34(4): 315-319.

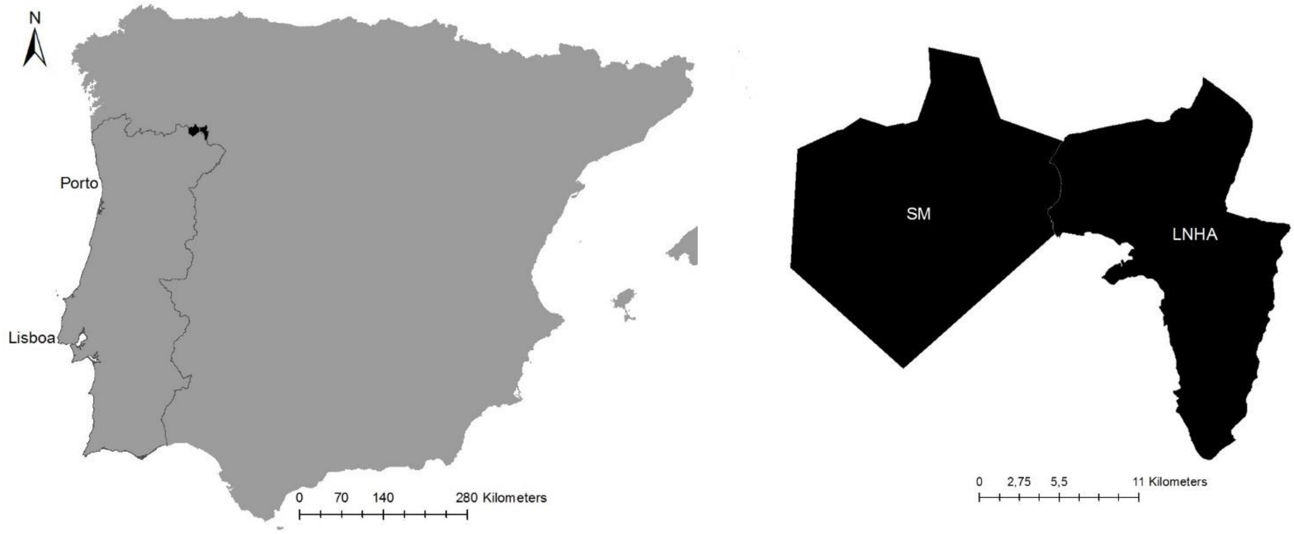

Figure 1. Location of the study area in the Iberian Peninsula. On the right there is the two study sites: **LNHA** – *Lombada National Hunting Area* and **SM** – *Serra de Montesinho*.

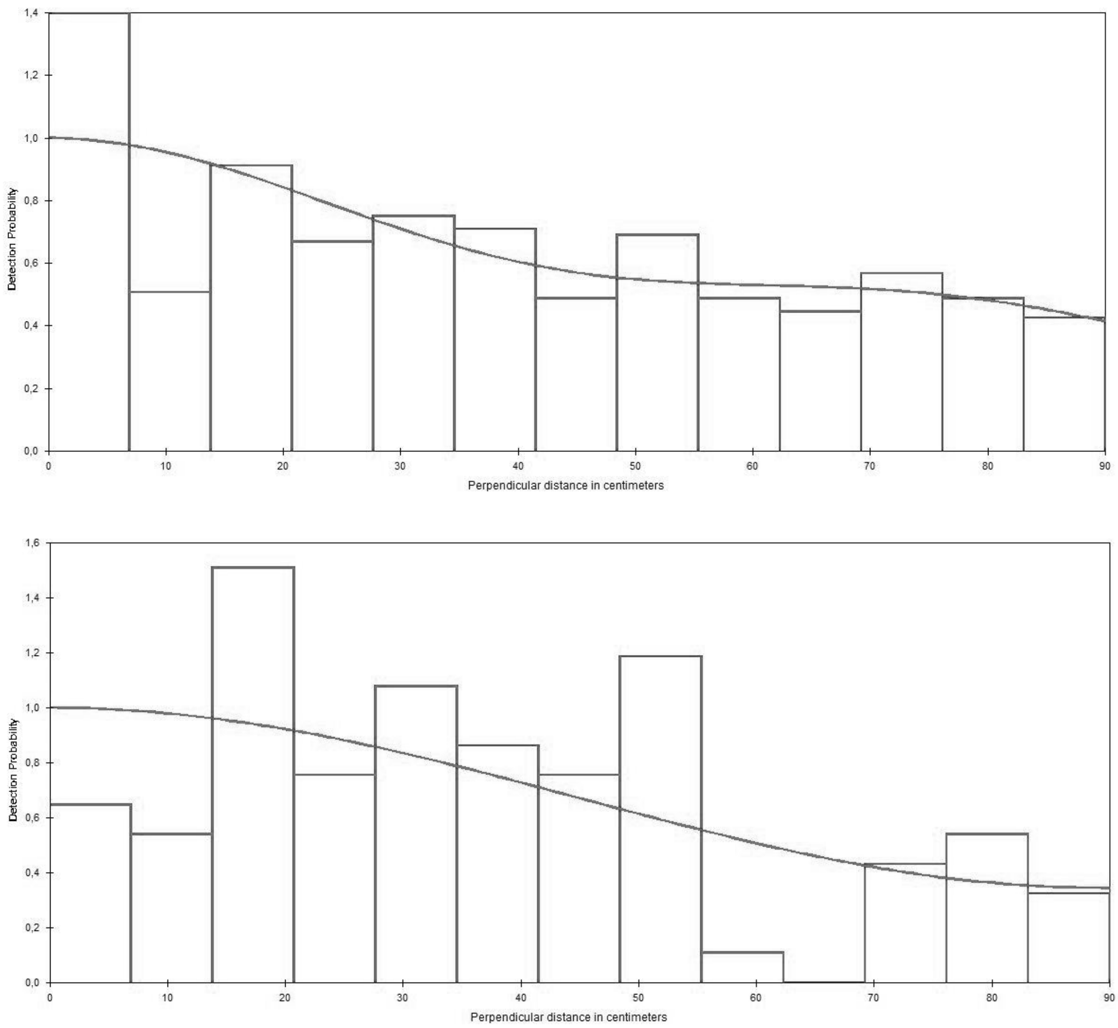

Figure 2. Stratified detection function of the distance data for LNHA and SM using a half-normal and a uniform model key function respectively and a cosine adjustment term in both analyses. Observed distances were right-truncated to eliminate the largest 5% of the distances. The model was fitted to continuous data, not binned data, and hence the histogram bars cannot be interpreted as probabilities.

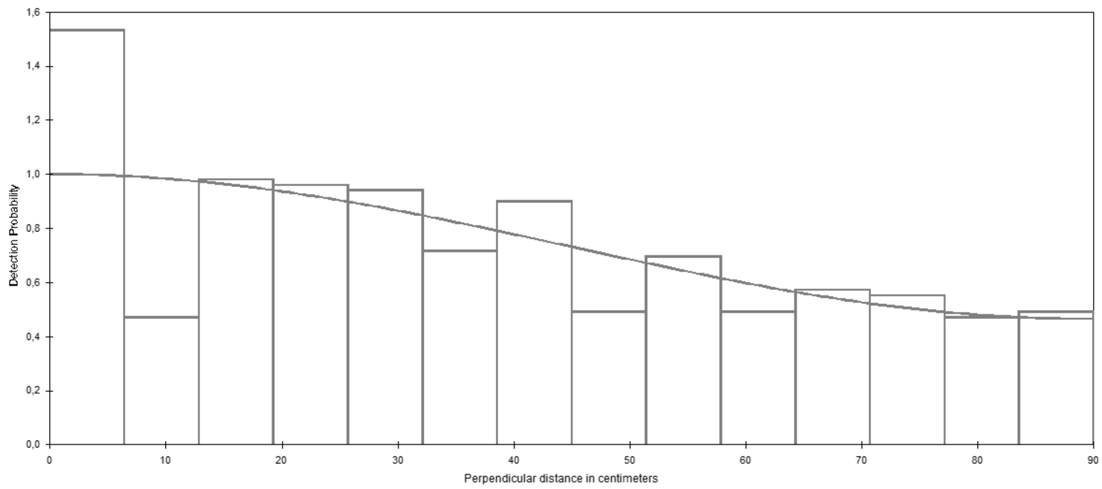

Figure 3. Pooled detection function of the distance data for survey area using a uniform model key function and a cosine adjustment term. Observed distances were right-truncated to eliminate the largest 5% of the distances. The model was fitted to continuous data, not binned data, and hence the histogram bars cannot be interpreted as probabilities.

Table 1. Summary statistics for the detection function models considered: AIC, ΔAIC and P-values associated with the χ^2 and Cramer von-Mises goodness-of-fit **(**CvM) tests.

Detection function	AIC	ΔAIC	Chi-squared goodness-of-fit	CvM
Individual – LNHA	3762.19		0.008	0.010
Individual – SM	721.25		0.035	0.400
Stratified	**4483.44***	0.00		
Pooled	4484.36	0.92	0.0007	0.010
Covariate Size	4488.37	4.93	0.00	0.010
Covariate Shape	4488.46	5.02	0.00	0.010
Covariate Detectability	4488.18	4.74	0.00	0.010
Covariate Habitat	4487.70	4.26	0.00	0.010

The "**Stratified**" summarize the two individual analyses: **LNHA** – Lombada National Hunting Area; **SM** – *Serra de Montesinho*. Note the χ^2 outputs of software Distance are based on a smaller number of bins for the CDS analysis than for the MCDS analysis.

*This value represents the sum of the two previous individual analyses.

Table 2. Red deer density, abundance and 95% IC in total area, LNHA and SM.

	Area (ha)	Total effort (m)	Density (per 100 ha)	Density (95% IC)		Density CV (%)	Abundance	Abundance (95% CI)	
Total area	45,600	19,600	3.38	2.18	5.24	22.18	1544	997	2391
LNHA	20,800	10,800	5.81	3.65	9.25	23.49	1211	761	1927
SM	24,800	8,800	1.34	0.74	2.42	29.61	333	185	599

Chapter III

A new insight for monitoring ungulates:

Density Surface Modelling of roe deer in a Mediterranean habitat

A new insight for monitoring ungulates: Density Surface Modelling of roe deer in a Mediterranean habitat

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3.1. Abstract

Ungulates are especially difficult to monitor and population estimates are difficult to obtain, however such information is fundamental for any effective management plan. This is particularly important for an expanding species as roe deer (*Capreolus capreolus*), whose populations have grown in number and distribution throughout the last decades. In an attempt to follow population fluctuations and assess species ecology, important methodological advances were recently achieved by combining line or point sampling with Geographic Information Systems (GIS). In this study a density surface modelling (DSM) approach was taken, combining line transect survey with spatial analysis to predict density of roe deer in north-eastern Portugal. This was based on the relationship of pellet group counts to environmental factors, as well as taking into account the probability of detecting pellets. It is the first time that such an approach is applied for an ungulate species in Europe. Our results showed a global density of 3.01 animals/100 ha (95% CI: 2.34 - 3.87) and a CV of 12.95%. Roe deer densities increased as distance to roads increased and decreased as distance to human populations increased. Densities also increased as the percentage of cover areas increased. This recently developed spatial method can be very advantageous to predict density over space through the identification of the factors influencing species abundance. Furthermore the drawing of surface maps for subset areas will enable to visually depict abundance distribution of wild populations. This will enable the assessment of areas where ungulate impacts should be minimized, allowing an adaptive management throughout time.

Keywords: *Capreolus capreolus*, density surface modelling, generalized additive models, Iberian Peninsula, spatial models.

3.2. Introduction

Large herbivores are particularly difficult to monitor (Schroeder et *al.* 2014) and ecologists have continuously sought more precise methodologies to do so. Successful strategies for the management of wide-ranging species require reliable information on abundance and population trends (Segura et *al.* 2007). Effective monitoring programs are pivotal to cope with the expansion of ungulate populations in Europe and North America over the last decades (Rooney 2001; Apollonio et *al.* 2010). Over the years significant efforts have been made to improve the methods used for monitoring wild populations (Buckland et *al.* 2001; Hedley & Buckland 2004; Thomas et *al.* 2010). *Distance sampling* (Buckland et *al.* 2001) has been widely used for estimating abundance and density of a variety of taxa such as birds (Marques et *al.* 2007), cetacean (Barlow & Forney 2007), small mammals (Newey et *al.* 2003) and ungulates (Marques et *al.* 2001; Focardi et *al.* 2005; Acevedo et *al.* 2010; Valente et *al.* 2014), and it has been shown to be a reliable and robust method (Marques et *al.* 2001; Acevedo et *al.* 2008). Sampling design consists in line or point transects where the animals or animal signs are searched: for each observation/detection, the perpendicular distance from the transect is recorded and a detection function is built enabling the abundance and density estimation of the population of interest. Nevertheless, with the fast advance of the spatial analysis techniques, the combination of spatial modelling with Geographic Information Systems (GIS) on population density estimation has been recently developed. This was firstly reviewed by Buckland et *al.* (2000), Hedley et *al.* (2004) and Hedley & Buckland (2004) who developed methods for improving abundance estimation of wildlife taking into account population's spatial distribution. This has allowed to account for heterogeneity in the population spatial distribution while accounting the probability of detecting the animal or its sign. Undoubtedly the relation between animal density and spatial variables is a step forward in ungulate management as mapping the spatial abundance distribution of a population can be extremely useful to wildlife managers, particularly when communicating results to non-experts stakeholders (Katsanevakis 2007; Miller et *al.* 2013a). Recently, density surface models (DSM) were developed enabling the identification of meaningful ecological (spatial) variables that can affect (constrain or uphold) animal populations densities (Katsanevakis 2007; Miller et *al.* 2013a). DSM offer a robust estimation of abundance (Katsanevakis 2007; Katsanevakis & Thessalou-Legaki 2009) and are simple to integrate with line transect framework of *distance sampling*. Furthermore such models do not require a restrictive survey design nor a uniform habitat coverage. Likewise this method has the advantage of allowing the estimation of abundance in sub-areas of interest, through numeric integration under the section of the fitted density surface (Katsanevakis 2007). This spatial methodology can also improve management plans, since it makes possible to identify subtle impacts on species, by estimating spatial redistribution of animals as a result of a particular hazard (Petersen et *al.* 2011). DSM are a model-based approach corrected for uncertain detection via a *distance sampling* framework (Hedley & Buckland 2004; Miller et *al.* 2013a), being typically implemented via generalized additive models (GAMs) (Hastie & Tibshirani 1990). DSM have been successfully implemented in a few species, *e.g.* aquatic molluscs (Katsanevakis 2007; Katsanevakis & Thessalou-Legaki 2009), marine mammals (Henrys 2005; Burt & Paxton 2006) and only recently to ungulate species (Schroeder et *al.* 2014).

The European roe deer (*Capreolus capreolus*) is the most abundant and widespread cervid species in Europe, with an estimated population of 10 million individuals (Apollonio et *al.* 2010). In the Iberian Peninsula, namely in Portugal, the southwestern limit of its distribution, roe deer shows low densities (Valente et *al.* 2014) particularly when compared with central and northern Europe (Apollonio et *al.* 2010). In Portugal, roe deer presence is more likely in areas with high shrub density and where red deer density and human presence is low (Torres et *al.* 2012a; Torres et *al.* 2012b). However both spatial heterogeneity and proximity to roads negatively influence its presence (Torres et *al.* 2011; Torres et *al.* 2012a). Following the current European trend, roe deer density is expected to dramatically increase in Portugal and it is timely to implement management strategies that can prevent the potential negative impacts that this species can have in ecosystems (for a review see Putman et *al.* 2011).

In the present study we combined line transect sampling with spatial analysis to predict the abundance of roe deer in northeastern Portugal. The abundance predictions were based on the relationship of pellet group counts to environmental factors, taking into account the probability of detecting pellets. This was done through the collection of distance data regarding pellet groups along line transects covering the whole survey area. Additionally, design-based and model-based approaches were compared to assess the potential advantages of spatial modelling in estimating this ungulate's abundance. DSMs used in our work should improve the accuracy of density and abundance estimates when compared with traditional *distance sampling*, since they model part of the spatial variability (Hedley et *al.* 2004). As far as we know, this model-based approach has never been used before for an European ungulate species, neither with an indirect approach (*e.g.* pellet group counting) and could be proved a very advantageous way of estimating abundance of wide-ranging species. This study has two main aims: i) estimate roe deer densities and abundance with the production of a roe deer abundance distribution map across the study area; ii) reveal the relationships between roe deer abundance and spatial covariates, identifying the factors involved in this species abundance and density. This work is part of a continued monitoring program of northeastern ungulate population.

3.3. Methods

3.3.1. Study area

The study was carried out in northeast Portugal (Montesinho Natural Park – MNP – and *Serra da Nogueira* – SN) (6º30'-7º12'W, 41 º43'-41 º59'N and 6º50'-6º56'W, 41º38'-41º48'N respectively), part of the European Union's Natura 2000 Network, covering an area of 63,500 ha (Figure 1). The terrain consists of rolling hills with elevation ranging from 438 to 1,481m. The climate is mainly Mediterranean with the mean annual temperature varying between 3ºC in the coldest month and 21ºC in the warmest month and precipitation varying between 600 mm and 1,500 mm (Castro et *al.* 2010). The vegetation is diverse, characterized mainly by oak (*Quercus pyrenaica*, *Q. rotundifolia*, *Q. suber*), sweet chestnut (*Castanea sativa*) and maritime pine (*Pinus pinaster*). The shrub vegetation is dominated by heather (*Erica* spp.), gum rockrose (*Cistus ladanifer*) and furze (*Ulex europaeus* and *Ulex minor*). Other mammals present are the Iberian wolf (*Canis lupus signatus*), red fox (*Vulpes vulpes*), wild cat (*Felis silvestris*), wild boar (*Sus scrofa*) and the sympatric red deer (*Cervus elaphus*), among others. The study area is crossed by some rivers and includes small villages with a low human presence (9.5 people per km^2).

3.3.2. Line transects and field work

The survey area was divided in 3 geographic strata: *Serra de Montesinho* (SM: 24,400 ha), *Serra da Nogueira* (SN: 18,300 ha) and Lombada National Hunting Area (LNHA: 20,800 ha) (Figure 1). This was done to improve the precision of a final density estimate since we expected *a priori* different densities across areas (Valente et *al.* 2014). Transect location and orientation was randomly chosen, ensuring that they were representative of all habitat types in the study area. In total, 65 transects were placed: 22 transects in SM, 16 in SN and 27 in LNHA. Each transect was 1,000m long: in order to maximize spatial coverage and to mitigate sampling dependence, sampling plots were 100m long of effective sampling, spaced 200m (without prospection) along the line (with a total of 400m on effort *per* transect). Field work was performed from January 2012 to October 2013 (2012: January and November; 2013: January, February and October). Using a handheld Global Positioning System (GPS) unit and a compass it was possible to follow a straight line. A rope was used to facilitate the progress in a straight line, ensuring the prospection of 1 meter from each side of the line, and guaranteeing accurate measurements of the perpendicular distances. Whenever a pellet group was detected, the perpendicular distance from the centre of each pellet group to the transect line was recorded as well as covariates thought to influence detectability of pellets (Marques et *al.* 2007). Three observation level covariates were collected: i) the size of the pellet group (medium, between 10 to 40 individual pellets vs. large, more than 40 individual pellets); ii) dispersion of the group (aggregated vs. scattered); and iii) the type of habitat around the pellet group (open vs. close). To minimize the bias in the estimates we considered only pellet groups with ten or more individual pellets (produced at the same defecation event, identified for similar size, shape, texture and colour). This practice reduces the risk of counting one spread group as two pellet groups (Marques et *al.* 2001).

3.3.3. A two-stage approach:

3.3.3.1. Modelling the detection function

The distance sampling approach relies on the assumption that the detection of animals/objects is not certain (Buckland et *al.* 2001; 2004). A detection function is used to model the decrease in detectability with increasing distance from the transect line (Buckland et *al.* 2001; Miller et *al.* 2013a). The detection function, *g(y)*, gives the probability of detecting an object in a distance *y* from the centre of the transect line. The probability of detection for the survey area can be estimated according to: $P = \int_0^w g(x)\pi(x)dx$

where w is a truncation distance and $\pi(x)$ represents the distribution of available distances, being assumed known to be uniform by design. In the first stage we used the *Distance* package (Miller 2014) in R (R Development Core Team 2013) to estimate roe deer density and abundance. Three key models were tested: uniform, half-normal and hazard-rate with the three adjustment terms available (cosine, simple polynomial and hermite polynomial). The role of the collected covariates in the detectability of pellet groups was assessed through Multiple Covariate Distance Sampling (MCDS) analysis (Marques et *al.* 2007). Model choice was based on the Akaike information criterion (AIC) choosing the one with the smallest value (Akaike 1974; Andersen et *al.* 1998). Additionally there was a visual inspection of the histogram of distance data and an analysis of goodness-of-fit tests (Burnham et *al.* 2004). Distance data were right-truncated to remove 5% of the perpendicular distances as recommended by Marques et *al.* (2001), resulting in a maximum width of 95 cm of effective prospection. Only the most parsimonious model was used for the density surface modelling.

3.3.3.2. Density surface modelling (DSM)

The second stage was also performed in R (R Development Core Team 2013) using the package *dsm* (Miller et *al.* 2013b). In this stage, it was necessary to split the 1,000 transect lines into segments of 100m, resulting in a total of 260 segments for the total surveyed area. Environmental covariates used were chosen based on roe deer ecological requirements and on their potential predictive capability, therefore variables were divided into: i) human disturbance (Hewison et *al.* 2001; Torres et *al.* 2011); ii) availability of cover areas (Virgós & Telléria 1998; Borkowski & Ukalska 2008). This is particularly important since roe deer represents one of the main prey for Iberian wolf, thus cover areas (coniferous and deciduous forests) are expected to have an important role acting as potential anti-predatory refuges (Borkowski & Ulkaska 2008; Torres et *al.* 2011). Accordingly, four transect level spatial covariates were collected through ArcMAP (version 10.1) and used to model the density surface of roe deer in our study area: i) geographic coordinates (latitude and longitude); ii) human disturbance variables (distance to the nearest road – dist_road – and distance to the near human settlement – dist_hum); iii) percentage of cover areas (ca_perc: coniferous and deciduous forests). The percentage of cover areas was extracted in a 1.26 km radius, which represents a home range scale, and was calculated based on home range values found in Portugal (Carvalho et *al.* 2008). We used GIS to build the buffers, which were built from the center of the 100m segments. The land cover information was obtained through CORINE Land Cover 2006 (CLC2006).

The models were built through stepwise backward elimination to sequentially simplify the full model, until only relevant terms and interactions remained (Crawley 2007). The count method of Hedley & Buckland (2004) was applied, using the number of pellet groups in each segment as the response variable in the density surface modelling, according to:

$$\mathrm{E}(n_j) = \hat{p}_j A_j \exp\left[\beta_0 + \sum_k f_k(z_{jk})\right]$$ (Miller et *al.* 2013a),

where z_{jk} is a sum of smooth functions of spatial covariates with *k* indexing the spatial covariates, f_k represents the smooth functions of the spatial covariates and β_0 is an intercept term. A_j is the segment area and $\hat{p}_j$ the detection probability (however, if this parameter is constant throughout the segments, $\hat{p}_j$ will be replaced by $\hat{p}$). The number of pellets (response variable) for each segment was related to the predictor variables through Generalized Additive Models (GAMs) (Hastie & Tibshirani 1990): a quasipoisson distribution and a logarithmic link function were used. The optimum degree of smoothing was defined through Generalized Cross Validation (GCV) score. By default *dsm* package applies a factor $\gamma = 1.4$ to model the effective degree of freedom in the GCV score to avoid overfitting (Miller et *al.* 2013b). The choice of the density surface model among the set of candidates was based on the lowest GCV value (Wood 2006).

3.3.4. Abundance estimation

For data analysis simplification a prediction grid with 635 square cells of 100 ha each was built in ArcMAP (version 10.1). The abundance of roe deer in the study area was estimated as the sum of the estimated abundance in each one of the grid cells, $E[\hat{n}_r]$, $\hat{N} = \sum_r E[\hat{n}_r]$, relying on the spatial model chosen for inference. Based on the predictions inferred by the model, and taking into account the value of each variable in each grid cell, an abundance map for the survey area was drawn in R (R Development Core Team 2013). To estimate the abundance two conversion factors were used: i) the defecation rate, estimated by Torres et *al.* (2013) to our study area and to the species of interest (176 ± 31 days) and ii) the production rate, which was considered to be 20 pellet groups *per* day (Mitchell et *al.* 1985). These values were embedded in the offset value to convert pellet groups density to animal's density, to allow a straightforward interpretation of the results. Variance for the abundance estimates of DSM analysis was obtained through the variance propagation method described by Williams et *al.* (2011). This approach enables a prompt estimation of variance value for the global analysis and for each sub-area.

3.4. Results

3.4.1. The first stage: Modelling the detection function

In a total of 26000m on effort (SM – 8800m; LNHA – 10800m; SN 6400m) a total of 365 pellet groups were recorded. The model that better fitted the distance data among the set of candidates was the uniform key function with 1 cosine adjustment term (Figure 2). As expected under the distance sampling theory, the probability of detecting pellet groups decreased with the increasing distance from the transect line, presenting however a peak at the 0.2-0.25 m, and a broad shoulder with a large amount of observations right on the transect line (Figure 2). The three models that included covariates in the analysis had less support from the data, thus were discarded for the subsequent analysis. The probability of detection for the chosen model was $\hat{p}$ = 0.623±0.026 SE. This analysis revealed an estimate of density of 3.53 animals *per* 100 ha (95% IC: 2.78 – 4.47), with a $\hat{N}$ = 2, 233 animals, and a coefficient of variation of 11.93% (Table 1).

3.4.2. The second stage: Density surface modelling

From all the candidate models, two were selected based on their GCV score (model 1 and model 3) (Table 2) for a DSM analysis. There was an implementation and development of two models in order to fully exploit the data: a model for the analysis of environmental data (explanatory model – model 1 - with dist_hum, dist_road and ca_perc spatial covariates), and a model that enables a more robust estimate of abundance through the inclusion of geographical data (predictive model – model 3 - with dist_hum, ca_perc, latitude and longitude spatial covariates). Figure 3 shows the smoothed spatial covariates used in the explanatory model, being dist_hum the most important variable in the analysis as revealed by p-values

(Table 2). The inspection of the residuals of the two density surface models revealed an adequate fit of the data.

3.4.3. Abundance estimation and uncertainty analysis

According to the best density surface model (predictive model) the abundance of roe deer in our study area was estimated to be $\hat{N}$ =1,909 animals with a density of 3.01 animals *per* 100 ha (95% IC: 2.34 – 3.87) and a coefficient of variation of 12.95%. In accordance with the predictive model chosen for inference the distribution map of roe deer throughout the study area is shown in Figure 4.

When comparing the % CV's, a difference of about 1% is registered between traditional distance sampling and DSM (for the total area). In fact, for SN the CV (%) for the DSM analysis is substantially smaller than the corresponding traditional *distance sampling* analysis. The comparison of abundance, density, 95% confidence intervals and coefficient of variation (%) (throughout the total area and for the three sub-areas) between traditional *distance sampling* and density surface models can be found in Table 1.

3.5. Discussion

Wildlife managers and ecologists have continuously searched for accurate, unbiased and cost-effective methods to estimate species abundance, density and distribution. Such demand has been particularly difficult to achieve for large herbivores. Density surface models (DSM), by combining animal density spatial variation with traditional line transect surveys, opens new possibilities for this (Schroeder et *al.* 2014). Therefore, estimating densities and relating them to meaningful ecological variables is a step further on wildlife management.

DSM allowed us to describe spatial patterns in the species predicted distribution. Our results show that roe deer have higher densities in areas further away from roads. Previous authors have described the same pattern for this species (Hewison et *al.* 2001; Torres et *al.* 2012a; Torres et *al.* 2012b). Roads constitute a source of disturbance and can cause mortality events, which are increasing in some areas (Seiler 2004). Roe deer tendency to avoid roads may be related to the risk of collision, which can jeopardize individuals life, as evidenced in elk (Rowland et *al.* 2000). Effectively, animals safety are more preserved further away from roads, and roe deer spatial distribution reflects this.

At first puzzling, our results show that roe deer densities increase in areas near human settlements. Our results do not corroborate previous studies (Hewison et *al.* 2001; Coulon et *al.* 2008), including a recent study in our study area (Torres et *al.* 2012b). Nevertheless, the two studies present important methodological differences that can explain these results. Torres et *al.* (2012b) used presence/absence of roe deer pellet groups as an index of habitat use while our model estimates the specific values of density for each grid cell, using additional information and hence potentially more accurate. The increasing density in direction to human settlements can be explained by the demographic decrease experienced in Montesinho Natural Park throughout the last years (Afonso 2012), resulting in small villages with very low human density (*e.g.* Rio de Onor in LNHA with 76 inhabitants in 2011 according to INE (2012)). Effectively, this pattern was also found in Spain (Aragón et *al.* 1995; Acevedo et *al.* 2005), where deer populations were positively associated with areas with declining human population (Aragón et *al.* 1995). However, further studies are need to analyse if roe deer closeness to human settlements can be an anti-predator strategy. A similar effect has been already shown for roe deer and lynx in Norway, where roe deer in more disturbed areas seemed to benefit from human land use practices, potentially allowing them to escape from lynx predation (Basille et *al.* 2009). The advantages of being near human populations can also be linked to the closeness to agricultural fields providing continuous availability of high-quality food (Torres et *al.* 2012a) as it occurs in some areas (Panzacchi et *al.* 2010).

Our results also show that increased roe deer densities correspond to areas with high percentage cover. This suggests the importance of these areas, especially when referring to prey populations (Borkowski & Ulkaska 2008) as roe deer in northeastern Portugal. Some studies (Smith et *al.* 1986; Mysterud & Østbye 1999) suggest that canopy cover functions as part of an anti-predator strategy, through the decrease in roe deer sightability and the possibility that scent spreads poorly in these habitats, reducing scent detection by Iberian wolf. Furthermore, roe deer preference for forested areas (*i.e.* cover areas) can also be due to a balance between the safety ensured by closed areas, and the food provided in such sites, which seems to be the best option in summer, when herbaceous layer becomes senescent (Bugalho & Milne 2003). Effectively, due to roe deer small size, this species are more dependent on the quality than on the quantity of resources (Demment & Soest 1985), which can explain the positive association with cover areas (Figure 3).

Besides the ecological relations, roe deer presents a particular regional distribution, unveiled through the inclusion of geographical data. The addition of geographical coordinates was made to minimize residual autocorrelations, improving the predictive power of the model and the deviance explained by spatial variables (Lobo & Martin-Piera 2002) (increasing the deviance explained in > 10% in comparison with the best explanatory model – model 1 in Table 2). At the same time the inclusion of geographical data enabled to draw a regional pattern in roe deer distribution: it is noticeable a distribution gradient heading north-south and east-west, being the highest density values registered in SM (Figure 4).

Valente et *al.* (2014) estimated a roe deer density in the same area of 3.51 ind./100ha (95% CI of 2.26 – 5.45). These authors have used the traditional *distance sampling* approach. Our results suggest that the density of roe deer estimated by DSM are 3.01 ind./100ha (95% CI 2.34 – 3.87). Although a decrease in the density has been registered (probably also due to the lowest CV registered in our work, narrowing the 95% CI), we should not make ecological conclusions, being essential a wider temporal scale to assess population fluctuations. However, it is worth noting the substantial decrease in the CV (%) value when applying the spatial methodology (from 22.08% in Valente et *al.* 2014 to 12.95% in our work).

In general, the underlying ecological assumptions of the models, as well as the surface map predicted, fits the data observed during the field survey and previous studies (Torres et *al.* 2011; Valente et *al.* 2014). Effectively, methodology performance is satisfactory for the chosen model (CV was 12.95%, which is lower than the 31% suggested by Focardi et *al.* 2006 as satisfactory CV value), which can be linked to the random sampling design employed, ensuring the cover of all representative habitats (Buckland et *al*. 2004), coupled with a model-based approach. Density obtained through DSM models was, generally, similar to that obtained through traditional *distance sampling*. Contrarily to the expected (Hedley et *al.* 1999; Forney 2000), the spatial models, by the inclusion of the spatial variables, did not increase, in general, the precision of the estimate, which can be due to the abovementioned well-designed field survey conducted in our work.

Reassuringly, the estimates obtained from different models did not varied widely (Table 2), indicating that predictions are robust to model choice. However, typically DSM results should be interpreted carefully due to GAMs flexibility (Wood 2006), which can yield unrealistic densities and surface maps through overfitting data or "edge effects" (Segura et *al.* 2007). Still, the first feature is partly addressed by the factor $\gamma = 1.4$ added to the analysis (Miller et *al.* 2013b) and the "edge effect" was not noticed during the inspection of the fits. It is worth noting that model selection in GAMs is still a developing research area (Hedley & Buckland 2004) and other indicators should be analyzed, during distance data spatial modelling (*e.g.* variables p-value). In our analysis, the p-value of the variables revealed the inexistence of a significant ecological variable ($p \leq 0.05$) for predictive models. Furthermore, the deviance explained in both models (model 1 with 7.17% and model 3 with 17.3%) was not satisfactory. This suggests the need of a future exploitation of other factors that can influence roe deer densities, such as interaction with the sympatric red deer. Additionally, a data analysis based on the sex and season should be assessed, in a future perspective, due to differences in male and female roe deer ecological requirements, and due to differences in resource availability throughout the year as shown for other deer species (Thirgood 1995). These goals can be achieved with direct methodologies, which should be linked to DSM in a near future for ungulate populations in Portugal.

Still, our results confirmed that the application of newsworthy method as DSM increases the potential of distance sampling data, and can be scientifically compelling since it can be applied to data collected in a cost-effective manner. This approach can be a valuable tool to assess species-environment relationships, and the use of these models should be encouraged to improve density predictive power, whenever spatial information is accessible. This work is part of a continued long-term monitoring program, representing a step forward in the application of recently developed distance sampling techniques, which aims to become the future in population size estimation and ecological assessment.

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3.7. References

Acevedo, P., Delibes-Mateos, M., Escudero, M. A., Vicente, J., Marco, J. & Gortázar, C. (2005) Environmental constraints in the colonization sequence of roe deer (*Capreolus capreolus* Linnaeus, 1758) across the Iberian Mountains, Spain. *Journal of Biogeography* 32.9. 1671-1680.

Acevedo, P., Ruiz-Fons, F., Vicente, J., Reyes-García, A. R., Alzaga, V., Gortázar, C. (2008) Estimating red deer abundance in a wide range of management situations in Mediterranean habitats. *Journal of Zoology*. 276(1):37–47.

Acevedo, P., Ferreres, J., Jaroso, R., Durán, M., Escudero, M. A., Marco, J. & Gortázar, C. (2010) Estimating roe deer abundance from pellet group counts in Spain: An assessment of methods suitable for Mediterranean woodlands. *Ecological Indicators* 10: 1226–1230.

Afonso, R. A. D. S. (2012) O Parque Natural de Montesinho e a promoção do desenvolvimento local. Tese de Mestrado em Planeamento Regional e Urbano, Universidade de Aveiro. Aveiro, Portugal.

Akaike, H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.

Apollonio, M., Andersen, R., Putman, R. (2010) European ungulates and their management in the 21st century. Cambridge: Cambridge University Press. 618 p.

Andersen, R., Duncan, P. & Linnell, J. D. C. (1998) The European roe deer: the biology of success. Scandinavian University Press, Oslo, Norway.

Aragón, S., Braza, F. & San José, C. (1995) Socioeconomic, physiognomic, and climatic factors determining the distribution pattern of roe deer *Capreolus capreolus* in Spain. *Acta Theriologica* 40, 37–43.

Barlow, J., & Forney, K. A. (2007) Abundance and population density of cetaceans in the California Current ecosystem. *Fishery Bulletin*, *105*(4), 509-526.

Basille, M., Herfindal, I., Santin-Janin, H., Linnell, J. D., Odden, J., Andersen, R., Høgda, K. A. & Gaillard, J. M. (2009) What shapes *Eurasian lynx* distribution in human dominated landscapes: selecting prey or avoiding people?. *Ecography*, *32*(4), 683-691.

Borkowski, J. & Ukalska, J. (2008) Winter habitat use by red and roe deer in pine-dominated forest. *Forest Ecology Management*, 255, 468-475.

Buckland, S. T., Goudie, I. B. J., & Borchers, D. L. (2000) Wildlife population assessment: past developments and future directions. *Biometrics*, *56*(1), 1-12.

Buckland, S. T., Anderson, D. R., Burnham, K. P., Laake, J. L., Borchers, D. L. & Thomas, L. T. (2001) Introduction to Distance Sampling. Oxford: Oxford University Press. 448 p.

Buckland, S. T., Anderson, D. R., Burnham, K. P., Laake, J. L., Borchers, D. L., & Thomas, L. (2004) Advanced Distance Sampling: Estimating Abundance of Wildlife Populations. London: Chapman & Hall. 446 p.

Bugalho, M. N., & Milne, J. A. (2003) The composition of the diet of red deer (*Cervus elaphus*) in a Mediterranean environment: a case of summer nutritional constraint? *Forest Ecology and Management*, *181*(1), 23-29.

Burnham, K. P., Buckland, S. T., Laake, J. L., Borchers, D. L., Marques, T. A., Bishop, J. R. B. & Thomas, L.. (2004) Further topics in distance sampling Advanced Distance Sampling, Oxford University Press. pp. 307–392.

Burt, M. L., & Paxton, C. G. M. (2006) Review of density surface modelling applied to JARPA survey data, 1–5.

Carvalho, P., Nogueira, A.J.A., Soares, A.M.V.M. & Fonseca, C. (2008) Ranging behaviour of translocated roe deer in a Mediterranean habitat: seasonal and altitudinal influences on home range size and patterns of range use. *Mammalia*, 72, 89-94.

Castro J. F. T., Fonseca F., Castro J. P., Nobre S. & Pires, L. C. (2010) Montesinho Natural Park: General description and natural values. Natural heritage from east to west: Case studies from 6 EU countries. New York: Springer. pp. 119–132.

Coulon, A., Morellet, N., Goulard, M., Cargnelutti, B., Angibault, J. M., & Hewison, A. M. (2008) Inferring the effects of landscape structure on roe deer (*Capreolus capreolus*) movements using a step selection function. *Landscape Ecology, 23*(5), 603-614.

Crawley, M. J. (2007) Statistical modelling. *The R Book, Second Edition*, 388-448.

Demment, M. W. & Van Soest, P. J. (1985) A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. *American Naturalist*, 641-672.

Focardi, S., Montanaro, P., Isotti, R., Ronchi, F., Scacco, M. & Calmanti, R. (2005) Distance sampling effectively monitored a declining population of Italian roe deer *Capreolus capreolus italicus*. *Oryx*, 39: 421–428.

Focardi, S., Aragno, P., Montanaro, P. & Riga, F. (2006) Inter-specific competition from fallow deer *Dama dama* reduces habitat quality for the Italian roe deer *Capreolus capreolus italicus*. *Ecography*, 3: 407–418.

Forney, K. A. (2000) Environmental models of cetacean abundance: reducing uncertainty in population trends. *Conservation Biology*. 14(5): 1271–1286.

Hastie, T. J. & Tibshirani, R. J. (1990) Generalized Additive Models. London: Chapman and Hall. 337 p.

Hedley, S. L. & Buckland, S. T. (2004) Spatial models for line transect sampling. *Journal of Agricultural, Biological and Environmental Statistics* 9:181–199.

Hedley, S. L., Buckland, S. T. & Borchers, D. L. (1999) Spatial modelling from line transect data. *Journal of Cetacean Research Management* 1(3): 255–264.

Hedley, S. L., Buckland, S. T. & Borchers, D. L. (2004) Spatial distance sampling models. *Advanced distance sampling: estimating abundance of biological populations.* New York: Oxford University Press. 48-70.

Henrys, B. P. (2005) Spatial distance sampling modeling of cetaceans observed from platforms of opportunity Dissertation M.Sc. Thesis. University of St. Andrews. p. 52.

Hewison, A. J., Vincent, J. P., Joachim, J., Angibault, J. M., Cargnelutti, B. & Cibien, C. (2001) The effects of woodland fragmentation and human activity on roe deer distribution in agricultural landscapes. *Canadian Journal of Zoology*, *79*(4), 679–689.

Instituto Nacional de Estatística, I. P. (2012) Censos - Resultados definitivos. Portugal – 2011. Lisboa, Portugal. 560 p.

Katsanevakis, S. (2007) Density surface modelling with line transect sampling as a tool for abundance estimation of marine benthic species: The *Pinna nobilis* example in a marine lake. *Marine Biology,* 152: 77–85.

Katsanevakis, S. & Thessalou-Legaki, M. (2009) Spatial distribution, abundance and habitat use of the protected fan mussel *Pinna nobilis* in Souda. *Aquatic Biology,* 8: 45–54.

Lobo, J. M., & Martín-Piera, F. (2002) Searching for a predictive model for species richness of Iberian dung beetle based on spatial and environmental variables. *Conservation Biology*, *16*(1), 158-173.

Marques, F.F. C., Buckland, S. T., Goffin, D., Dixon, C. E., Borchers, D. L., Mayle, B. A., & Peace, A. J. (2001) Estimating deer abundance from line transect surveys of dung: sika deer in southern Scotland. *Journal of Applied Ecology*, 38: 349–363.

Marques, T. A., Thomas, L., Fancy, S. G. & Buckland, S. T. (2007) Improving estimates of bird density using multiple-covariate distance sampling. *Auk* 124: 1229–1243.

Miller, D. L., Burt, M. L., Rexstad, E. A., & Thomas, L. (2013a) Spatial models for distance sampling data: recent developments and future directions. *Methods in Ecology and Evolution*, *4*(11), 1001–1010.

Miller, D. L., Rexstad, E., Burt, L., Bravingtion M. V., Hedley, S. (2013b) dsm: Density surface modelling of distance sampling data. R package version 2.1.3. URL: http://CRAN.R-project.org/package=dsm

Miller, D. L. (2014) Distance: A simple way to fit detection functions to distance sampling data and calculate abundance/density for biological populations. R package version 0.8.0. http://CRAN.R-project.org/package=Distance

Mitchell, B., Rowe, J. J., Ratcliffe, P., & Hinge, M. (1985) Defecation frequency in roe deer (*Capreolus capreolus*) in relation to the accumulation rates of faecal deposits. *Journal of zoology*, *207*(1), 1-7.

Mysterud, A., & Østbye, E. (1999) Cover as a habitat element for temperate ungulates: effects on habitat selection and demography. *Wildlife Society Bulletin*. 385-394.

Newey, S., Bell, M., Enthoven, S. & Thirgood, S. (2003) Can distance sampling and dung plots be used to assess the density of mountain hares *Lepus timidus*? *Wildlife Bioogy*, *9*(3), 185-192.

Panzacchi, M., Herfindal, I., Linnell, J., Odden, M., Odden, J., Andersen, R. (2010) Tradeoffs between maternal foraging and fawn predation risk in an income breeder. *Behaviour Ecological Sociobiology*. 64, 1267–1278.

Petersen, I. K., Mackenzie, M., Rexstad, E., Wisz, M. S., Fox, A. D. (2011) Comparing pre- and post-construction distributions of long-tailed ducks *Clangula hyemalis* at a danish offshore wind farm. Techichal Report 2011–1, Centre for Research into Ecological and Environmental Modelling, St Andrews.

Putman R., Watson, P. & Langbein, J. (2011) Assessing deer densities and impacts at the appropriate level for management: a review of methodologies for use beyond the site scale. *Mammal Review*: 197–219.

R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org

Rooney, T. P. (2001) Deer impacts on forest ecosystems: a North American perspective. *Forestry*, *74*(3), 201–208.

Rowland, M. M., Wisdom, M. J., Johnson, B. K., & Kie, J. G. (2000). Elk distribution and modeling in relation to roads. *The Journal of Wildlife Management*, 672-684.

Schroeder, N. M., Matteucci, S. D., Moreno, P. G., Gregorio, P., Ovejero, R., Taraborelli, P., & Carmanchahi, P. D. (2014) Spatial and seasonal dynamic of abundance and distribution of guanaco and livestock: insights from using density surface and null models. *PloS One*, *9*(1), e85960.

Segura, A. G., Hammond, P. S., Cañadas, A., & Raga, J. A. (2007) Comparing cetacean abundance estimates derived from spatial models and design-based line transect methods. *Marine Ecology Progress Series*, *329*, 289-299.

Seiler, A. (2004). Trends and spatial patterns in ungulate-vehicle collisions in Sweden. *Wildlife Biology*, *10*(4), 301-313.

Smith, H. D., Oveson, M. C., & Pritchett, C. L. (1986) Characteristics of mule deer beds. *Great Basin Naturalist*. 46, 542-546.

Thirgood, S. J. (1995) The effects of sex, season and habitat availability on patterns of habitat use by fallow deer (*Dama dama*). *Journal of Zoology*, *235*(4), 645-659.

Thomas, L., Buckland, S. T., Rexstad, E. A., Laake, J. L., Strindberg, S., Hedley, S. L. & Burnham, K. P. (2010) Distance software: design and analysis of distance sampling surveys for estimating population size. *Journal of Applied Ecology*, *47*(1), 5-14.

Torres, R. T., Santos, J. P., Linnell, J. D. C., Virgós, E. & Fonseca, C. (2011) Factors affecting roe deer occurrence in a Mediterranean landscape, Northeastern Portugal. *Mammal Biology*. 76: 491–497.

Torres, R. T., Virgós, E., Santos, J., Linnell, J. D. & Fonseca, C. (2012a) Habitat use by sympatric red and roe deer in a Mediterranean ecosystem. *Animal Biology*. *62*, 351-366.

Torres, R. T., Virgós, E., Panzacchi, M., Linnell, J. D. C. & Fonseca, C. (2012b) Life at the edge: Roe deer occurrence at the opposite ends of their geographical distribution, Norway and Portugal. *Mammal Biology,* 77: 140–146.

Torres, R. T., Santos, J. P. & Fonseca, C. (2013) Persistence of roe (*Capreolus capreolus*) and red (*Cervus elaphus*) deer pellet-groups in a Mediterrenean mosaic landscape. *Mammal Biology,* 9: 7–18.

Valente, A. M., Fonseca, C., Marques, T. A., Santos, J. P., Rodrigues, R. & Torres, R. T. (2014) Living on the edge: roe deer (*Capreolus capreolus*) density in the margins of its geographical range. *PloS One*, *9*(2), e88459.

Virgós, E. & Telléria, J. L. (1998) Roe deer habitat selection in Spain: constraints on the distribution of a species. *Canadian Journal of Zoology*. 76, 1294-1299.

Williams, R. O. B., Hedley, S. L., Branch, T. A., Bravington, M. V., Zerbini, A. N. & Findlay, K. P. (2011) Chilean blue whales as a case study to illustrate methods to estimate abundance and evaluate conservation status of rare species. *Conservation Biology*, *25*(3), 526-535.

Wood, S. N. (2006) Generalized Additive Models: An introduction with R. Florida: Chapman and Hall/CRC. 410 p.

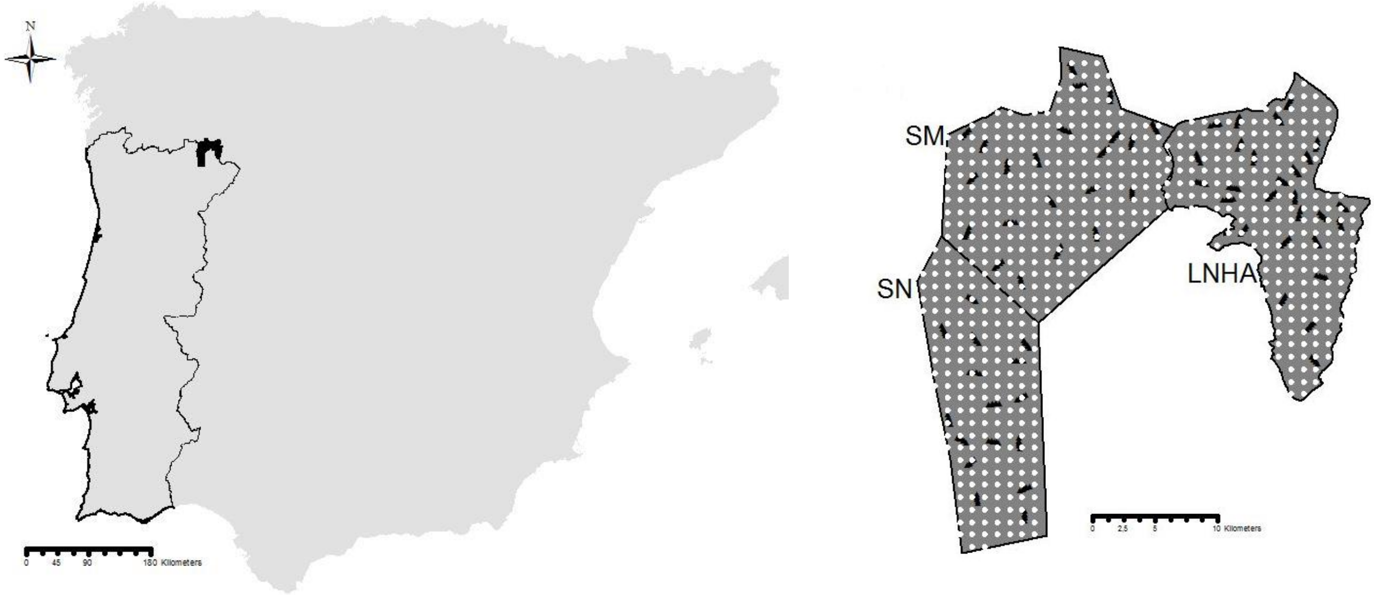

Figure 1. Location of the study area in the Iberian Peninsula with transects location and prediction grid in the survey area (**SN** – *Serra da Nogueira*; **SM** – *Serra de Montesinho*; **LNHA** – Lombada National Hunting Area).

Figure 2. Histogram of distance data of uniform model with cosine adjustment term. Observed distances were right-truncated to eliminate the largest 5% of the distances. The model was fitted to continuous data, not binned data, and hence the histogram bars cannot be interpreted as probabilities.

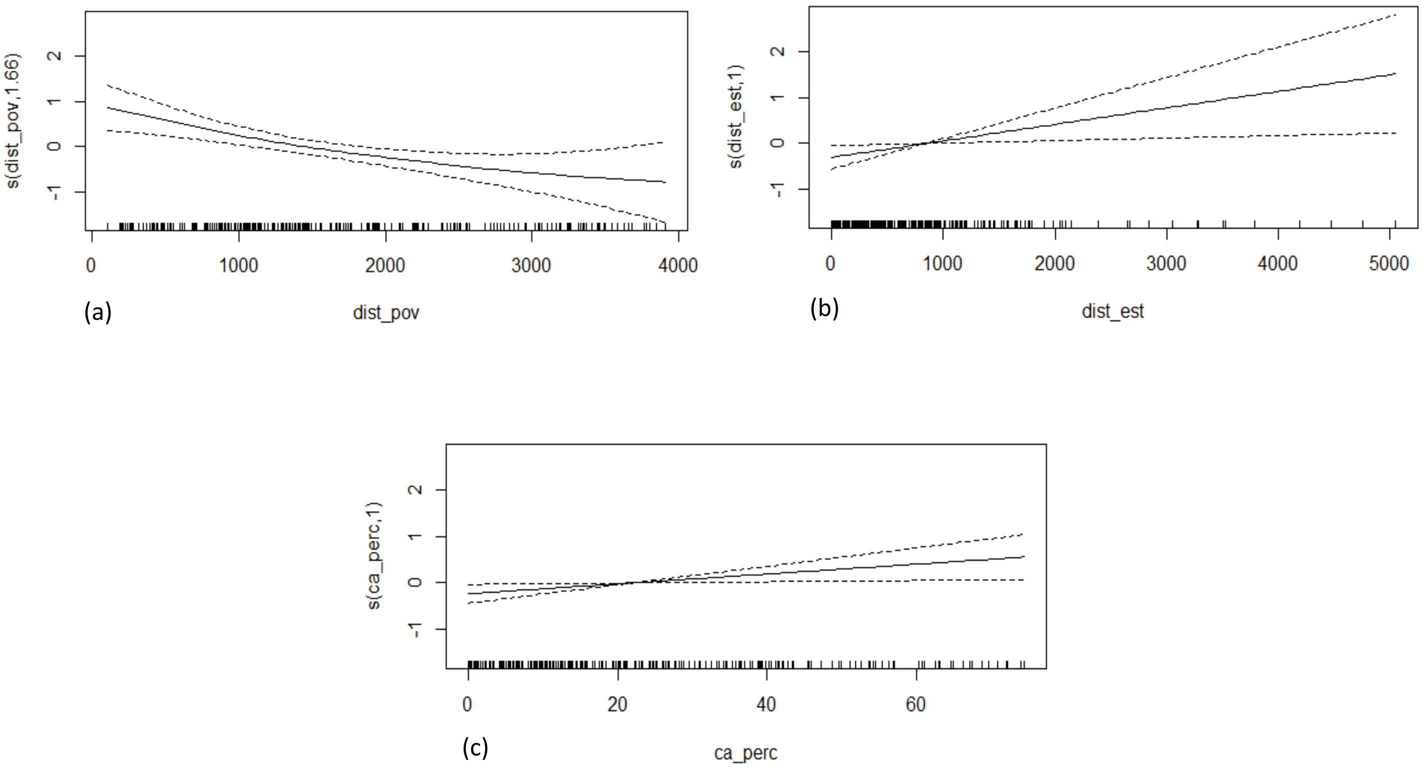

Figure 3. Shape of the functional forms of smoothed spatial covariates with the explanatory model – **(a) dist_hum** representing the distance to the nearest human settlement; **(b) dist_road** representing the distance to the nearest road and **(c) ca_perc** representing the percentage of cover areas (coniferous and deciduous forests).

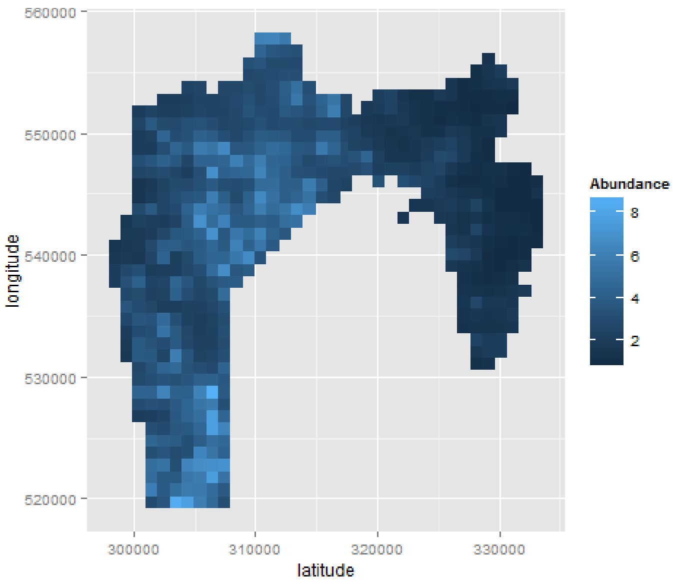

Figure 4. Abundance distribution map of roe deer throughout our study area based on the predictive model chosen for inference (model 3).

Table 1. Comparison between Density Surface Model and traditional *distance sampling* through analysis of abundance, density, 95% Confidence Interval and Coefficient of Variation (%) for the total area and for the three sub-areas: SN, SM and LNHA.

	Abundance estimates							
Method	**DSM (predictive mod.)**	**DS**	**DSM (predictive mod.)**	**DS**	**DSM (predictive mod.)**	**DS**	**DSM (predictive mod.)**	**DS**
	Total area		**SN**		**SM**		**LNHA**	
Abundance	1,909	2,233	662	693	913	1,262	331	278
Density	3.01	3.53	3.62	3.79	3.74	5.17	1.59	1.34
Density - 95% Confidence Interval	2.34 – 3.87	2.78 – 4.47	2.59 – 5.04	2.22 – 6.46	2.74 – 5.10	3.88 – 6.89	1.05 – 2.42	0.90 – 1.99
Coefficient of variation (%)	12.95	11.93	17,08	25.63	15.95	13,99	21.55	19.57

Table 2. Comparison between GCV score, R-square (adjusted), deviance explained, coefficient of variation (CV) and abundance among explanatory and predictive models, with comparison of p-values of each variable.

	p-value	GCV	R-square (adjusted)	Deviance explained (%)	CV (%)	Abundance
Explanatory model						
Model 1 *		**2.694**	**0.047**	**7.17**	**13.17**	**1877**
dist_hum	**0.003**					
dist_road	**0.019**					
ca_perc	**0.022**					
Predictive model						
Model 2		2.561	0.106	17.4	13.03	1926
dist_hum	0.096					
dist_road	0.577					
ca_perc	0.084					
geographic	0.030					
Model 3 *		**2.535**	**0.108**	**17.3**	**12.95**	**1909**
dist_hum	**0.105**					
ca_perc	**0.067**					
geographic	**0.008**					
Model 4		2.554	0.082	13.9	12.87	1836
ca_perc	0.120					
geographic	0.003					
Model 5		2.552	0.076	13.1	12.91	1846
geographic	0.002					

*model chosen for inference.

Chapter IV

Final considerations

4. Final considerations

Monitoring wild ungulate populations should be the first step of a management program based on scientific data collected over the years. We hope that this work will serve as base-line for ecological and methodological studies regarding ungulates in Mediterranean habitats. Throughout the thesis, all the major aims, both methodological and ecological, were achieved: density and abundance estimates for red and roe deer were obtained (Chapter II and III respectively) with a *distance sampling* approach in all analysis; traditional *distance sampling* and density surface models were able to provide straightforward density and abundance estimates with acceptable coefficients of variance, both for red (Chapter II) and roe deer (Chapter III).

Ecologically, our results showed that human disturbance does not always affect negatively roe deer. Although roe deer densities increased as distance to roads increased as well, their densities increased as we moved closer to human settlements. Still, these results should be further investigated to understand in-depth the reasons which led roe deer to areas closer to human populations (for a detailed discussion see Chapter III). As expected, cover areas also revealed its importance in roe deer ecological requirements.

In terms of density, red deer increased throughout our study area (Santos 2007; Santos 2009; Carvalho 2011), following the European trend (Apollonio et *al.* 2010). When comparing red and roe deer densities in overlapping areas (SM and LNHA), it is possible to notice a contraposition of results, *i.e.* in LNHA, where red deer population nuclei have been formed (Santos 2009), and where their densities reach higher values (5.81 ind./100 ha), roe deer densities tend to be substantially smaller (with 1.59 ind./100 ha). In SM, where roe deer densities are higher (3.74 ind./100 ha), red deer population size decrease notoriously (with 1.34 ind./100 ha). This is in agreement with previous studies (Torres et *al.* 2012). In fact, in SN, where roe deer densities are estimated to be 3.62 ind./100 ha, red deer presence was not yet confirmed. A deeper analysis on the relation between these sympatric species should be assessed in order to perform management plans that take into account the factors that can limit or uphold both species range and number. Furthermore, the influence of the presence/absence and density of Iberian wolf, roe and red deer main predator, should be assessed in future works. It might be interesting to include the presence/density of a species as an explanatory variable for the presence/density of other species in a DSM context.

Although our work represents an important step regarding roe deer ecological requirements, our results also show the urgent need to assess other factors which can potentially affect positively or negatively species abundance, since our spatial variables could not explain species distribution as desired (the best model with 17.3% of explained deviance). Effectively, DSM has the main drawback of having to know *a priori* which factors constrain or uphold species densities, or alternatively include a wide number of spatial variables, which can be time-consuming and some important information may not be accessible.

Through the inclusion of variables that can affect species densities, DSMs are one step ahead. Even when the CV values do not reflect this (such as in Chapter III where DSM CV value for the total area was higher than with traditional DS), the spatial approach capabilities lead this methodology to a promising future in wild population density estimates and ecological assessment. Bearing this, DSMs are able to address a shortcoming in conventional *distance sampling* derived estimates: the fact that it only provides wildlife managers with a simple number for density/abundance. DSM has opened new opportunities to perform data analysis based on spatial data that can yield important ecological information, as in this work, while collecting data with low human, economic and logistical resources. That being said it does not imply that traditional *distance sampling* is discouraged. Actually, *distance sampling* proved its potential as a cost-effective and simple approach able to estimate ungulate population size in a robust manner. In fact it represents a less complex way of getting density values, and can be applied even when spatial information regarding species ecological requirements is not available.

From an ecological point of view, it is worth noticing the importance of both works (Chapter II and Chapter III) in a wider temporal scale, promoting a continued following of trends for two widespread ungulate species. Continued monitoring programs are particularly important for species whose densities can potentially cause several damages (for a review see Putman et *al.* 2011) that can only be handled with a previous knowledge of population size and ecological constraints. The methodologies used in this study, applied for species known to be difficult to monitor, will allow, in the long-term, an adaptation of management strategies, such as the adjustment of the hunting bags, in order to preserve ungulate populations while avoiding the conflicts with human populations (due to damages caused by species in agricultural fields (Putman & Moore 1998), and the increase in deer-vehicle collisions (Seiler 2004), including other factors). Furthermore, this thesis enhance the need to apply monitoring programs in other areas, where ungulates are known to be increasing. Throughout Chapters II and III, the methodological tools to achieve robust density estimates are explained and discussed, giving wildlife managers *a priori* knowledge about methods performance in Mediterranean habitats.

Population size estimation and ecological assessment achieved throughout our study should be integrated in a multidisciplinary effort, that must also assess other important factors regarding species and habitats as the monitoring of the effects of the densities and habitat use of ungulates on the vegetation; monitoring of the body condition of hunted animals; monitoring aspects such as diet composition, stress and parasite burdens through faecal analysis; and monitoring infectious disease prevalence. Effectively, only with a multidisciplinary perspective a well-based management plan can be achieved, in order to handle ungulates as a part of an ecosystem, which inevitably suffers the consequences of an increasing population. Bearing in mind the principles of adaptive management, assessing both population trends as well as individuals' and habitat conditions is essential to perform management practices according to the ecosystem needs.

This work attempts to unveil ecological information, essential for well-designed monitoring programs that should be applied continuously with robust methodologies. This will give rise to management plans that will aim to meet species ecological needs, also partly unveiled during this study. It is also essential to perceive these populations in a wider scale, enhancing shared management of transboundary deer populations in the Iberian Peninsula.

4.1. References

Apollonio, M., Andersen, R., Putman, R. (2010) European ungulates and their management in the 21st century. Cambridge: Cambridge University Press. 618 p.

Carvalho, J. (2011) Monitorização da população de veado (*Cervus elaphus* L.) na Zona de Caça Nacional da Lombada, Nordeste Transmontano: Época 2010/2011. Universidade de Aveiro. Aveiro.

Putman, R. J., & Moore, N. P. (1998) Impact of deer in lowland Britain on agriculture, forestry and conservation habitats. *Mammal Review*, 28(4), 141-164.

Putman, R., Watson, P. & Langbein, J. (2011) Assessing deer densities and impacts at the appropriate level for management: a review of methodologies for use beyond the site scale. *Mammal Review*: 197–219.

Santos, J. P. (2007) Dinâmica populacional do veado (*Cervus elaphus*) no Nordeste trans-montano. 63pp. Universidade de Aveiro. Aveiro.

Santos, J. P. (2009) Estudo populacional do veado (*Cervus elaphus* L.) no Nordeste Trans-montano. Universidade de Aveiro. Aveiro.

Seiler, A. (2004) Trends and spatial patterns in ungulate-vehicle collisions in Sweden. *Wildlife Biology*, *10*(4), 301-313.

Torres, R. T., Virgós, E., Santos, J., Linnell, J. D., & Fonseca, C. (2012) Habitat use by sympatric red and roe deer in a Mediterranean ecosystem. *Animal Biology*, *62*(2).